Linking Investigations in Trauma and Emergency Services

Task Order 0007

Type O Whole blood and assessment of AGE during prehospital Resuscitation (TOWAR) Trial

Protocol v8_8.23.2022

Contents

Protocol Synopsis

Protocol Title:	Type O Whole blood and assessment of AGE during prehospital Resuscitation (TOWAR) Trial
Protocol Number:	STUDY20110430
NCT Number:	NCT04684719
Version # and Date:	Version 8.0 dated 8/23/2022
Investigational Drug:	Low titer whole blood
Trial Sites:	Clinical Coordinating Center • University of Pittsburgh, University of Pittsburgh Medical Center, PI Jason Sperry, Co-PI Frank Guyette Additional Sites: • University of Alabama at Birmingham • University of Washington/ Harborview Medical Center • University of Tennessee Medical Center Knoxville • University of Texas Houston • University of Louisville • Vanderbilt University • University of Mississippi • University of Cincinnati • MetroHealth
Funding Agency	Department of Defense
IND Sponsor:	Jason L Sperry, MD, MPH
Study Aims:	**AIM#1:** Determine whether prehospital low titer whole blood as compared to standard prehospital resuscitation results in lower 30-day mortality in patients at risk of hemorrhagic shock. **AIM#2:** Determine whether prehospital whole blood (age > 14 days) as compared to young whole blood (age ≤ 14 days) is associated with equivalent clinical outcomes, hemostasis, prevention of coagulopathy, and platelet function in patients at risk of hemorrhagic shock. **AIM#3:** Determine whether prehospital low titer whole blood as compared to standard prehospital resuscitation results in lower early mortality, blood and blood component transfusion requirements, incidence of coagulopathy, improved hemostasis and platelet function in patients at risk of hemorrhagic shock.
Study Design:	Open label, multi-center, pre-hospital randomized trial utilizing 10 level-1 trauma centers designed to determine the efficacy and safety of low titer whole blood resuscitation as compared to standard of care

	resuscitation in patients at risk of hemorrhagic shock and to appropriately characterize the hemostatic competency of whole blood relative to its age.
Planned Sample Size:	1020 patients
Planned Study Time:	6-year, multi-center study; 4-year enrollment
Major Inclusion Criteria:	1.) Injured patients at risk of hemorrhagic shock being transported from scene or referral hospital to a participating TOWAR trial site that meet requirements for initiation of blood or blood component transfusion **AND** 2A.) Systolic blood pressure ≤90mmHg and tachycardia (HR ≥108) at scene, at outside hospital or during transport **OR** 2B.) Systolic blood pressure ≤ 70mmHg at scene, at outside hospital or during transport
Major Exclusion Criteria:	1. Wearing "NO TOWAR" opt–out bracelet 2. Age > 90 or < 18 years of age 3. Isolated fall from standing injury mechanism 4. Known prisoner or known pregnancy 5. Traumatic arrest with > 5 consecutive minutes of CPR without return of vital signs 6. Brain matter exposed or penetrating brain injury (GSW) 7. Isolated drowning or hanging victims 8. Objection to study voiced by subject or family member at the scene 9. Inability to obtain IV or intraosseous access 10. Isolated burns without evidence of traumatic injury
Primary Endpoint:	30-day mortality

I. Specific Aims

Resuscitation strategies for the acutely injured patient in hemorrhagic shock have evolved. Patients benefit from receiving less crystalloid and early red blood cell transfusion with balanced ratios of plasma and platelets.[1-3] These resuscitation practices are termed Damage Control Resuscitation and are incorporated into massive transfusion protocols in level 1 trauma centers across the country.[2-4] This reconstituted strategy has also been coined 'whole blood-like' resuscitation despite being inferior compositionally to whole blood.[5,6]

Despite these changes, deaths from traumatic hemorrhage continue to occur in the first hours following trauma center arrival, underscoring the importance of early interventions which provide benefit.[1-3,7] Due to the time sensitive nature of the treatment of hemorrhage, the ideal resuscitation intervention would entail use of blood products containing all essential hemostatic

components, administration closest to time of injury, and mitigation of the devastating downstream consequences of shock and coagulopathy.

Whole blood transfusion following traumatic injury represents the 'essential next step' for the management of hemorrhagic shock post-injury. Prehospital whole blood is significant in that it brings this lifesaving hospital intervention to those patients who need it most, at a time before hemorrhagic shock and coagulopathy begin to have their detrimental consequences. We hypothesize that the initiation of whole blood resuscitation in the prehospital setting will significantly reduce the morbidity and mortality attributable to hemorrhagic shock post-injury as compared to standard prehospital resuscitation practice. Thus, a large pragmatic clinical trial is needed to definitively establish the efficacy and safety of whole blood resuscitation initiated in the prehospital setting. Only a high-quality clinical trial will provide the evidence to justify the use of this limited resource early post-injury.

Whole blood is a precious resource. The shelf life of the whole blood product depends on separation procedures and ranges from 21 to 35 days. The storage of whole blood leads to platelet dysfunction, cell lysis, and release of potassium and free heme which may reduce the resuscitative capacity of whole blood or contribute to end organ injury. The safety and efficacy of whole blood as a resuscitation fluid as it approaches its shelf life remains poorly characterized. The aims of the current Type O Whole blood and assessment of Age during prehospital Resuscitation (TOWAR) trial are to determine the efficacy and safety of whole blood resuscitation as compared to standard care resuscitation in patients at risk of hemorrhagic shock and to appropriately characterize the hemostatic competency of whole blood relative to its age.

AIM#1: Determine whether prehospital low titer whole blood as compared to standard prehospital resuscitation results in lower 30-day mortality in patients at risk of hemorrhagic shock.

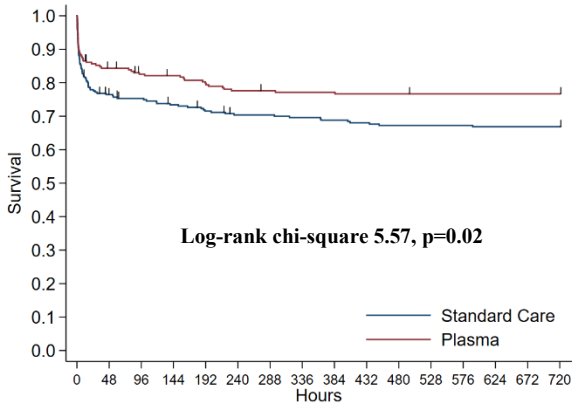
AIM#2: Determine whether old prehospital whole blood (age > 14 days) as compared to young prehospital whole blood (age ≤ 14 days) is associated with equivalent clinical outcomes, hemostasis, prevention of coagulopathy, and platelet function in patients at risk of hemorrhagic shock.

AIM#3: Determine whether prehospital low titer whole blood as compared to standard prehospital resuscitation results in lower early mortality endpoints, blood and blood component transfusion requirements, lower incidence of coagulopathy, and improved hemostatic and platelet function in patients at risk of hemorrhagic shock.

II. Background and Significance

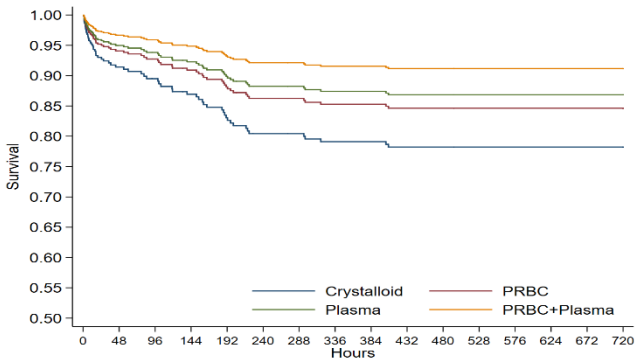
Initiation of the tenets of damage control resuscitation early, in the prehospital setting, has the potential to reduce downstream complications attributable to hemorrhage by intervening closer to the time of injury, prior to the development of coagulopathy, irreversible shock, and the ensuing inflammatory response.[8-11] Thawed plasma transfusion has been shown to safely reduce 30-day mortality when infused early, in the prehospital setting, in patients at risk of hemorrhagic shock and this separation of survival occurs within the first 3 hours.[12] (**Figure 1**.)

Figure 1. 30-day survival analysis



A recent secondary analysis from the PAMPer trial demonstrated that patients who received both plasma and red cells (an independent predictor of mortality in the cohort) in the prehospital setting, had the best adjusted survival. Patients receiving crystalloid resuscitation only, had the highest mortality.[13] (**Figure 2**.)

Figure 2. Pamper Prehospital Blood Component Secondary Analysis



We hypothesize that the initiation of low titer whole blood (LTWB O) resuscitation in the prehospital setting is the most effective resuscitation for those at risk of hemorrhage and significantly reduces the morbidity and mortality attributable to hemorrhagic shock post-injury as compared to standard prehospital resuscitation practice. A large pragmatic clinical trial is needed to definitively establish the efficacy and safety of whole blood resuscitation initiated in the prehospital setting and to characterize its effectiveness as a function of storage time. Only a high-quality clinical trial will provide the evidence to justify the use of this precious blood banking resource early post-injury.

Study Design/Setting: The current proposed trial will be a 6-year (4-year enrollment), multi-center, open label, prehospital randomized trial utilizing 10 level-1 trauma centers from within the LITES network and will enroll approximately 1020 patients. The University of Pittsburgh will be the Clinical Coordinating Center and the Data Coordinating Center for the study.

Study Population**:** Injured patients at risk of hemorrhagic shock requiring blood transfusion in prehospital phase of care.

Inclusion Criteria:

1.) Injured patients at risk of hemorrhagic shock being transported from scene or referral hospital to a participating TOWAR trial site that meet requirements for initiation of blood or blood component transfusion

AND

2A.) Systolic blood pressure ≤ 90mmHg and tachycardia (HR ≥ 108) at scene, at outside hospital or during transport

OR

2B.) Systolic blood pressure ≤ 70mmHg at scene, at outside hospital or during transport

Exclusion Criteria:

1. Wearing NO TOWAR opt–out bracelet
2. Age > 90 or < 18 years of age
3. Isolated fall from standing injury mechanism
4. Known prisoner or known pregnancy
5. Traumatic arrest with > 5 consecutive minutes of CPR without return of vital signs
6. Brain matter exposed or penetrating brain injury (GSW)
7. Isolated drowning or hanging victims
8. Objection to study voiced by subject or family member at the scene
9. Inability to obtain IV or intraosseous access
10. Isolated burns without evidence of traumatic injury

Study Intervention: Two units of low titer whole blood, group O (LTWB O) will be collected using local site approved blood banking protocols and will be stored at each respective EMS base or stocking facility for each EMS unit/service. Low titer will be defined as anti-A and anti-B antibody titers <256 and only units meeting this threshold or lower will be issued for prehospital study use. Whole blood will be collected and kept to either 21 days or 35 days based upon which preservation process is employed at each respective participating site (CPD-21 days shelf life, CPDA-1-35 days shelf life).

Study Intervention Arm: Patients will receive up to two units of whole blood as collected by local blood bank procedures and stored at 1-6 degrees Celsius initiated in the prehospital phase of care when available once all inclusion criteria and no exclusion criteria are met. Once TOWAR trial site arrival occurs and prehospital initiated intervention transfusions are completed, randomized patients will receive standard in-hospital trauma care.

Standard Care Arm: Patients will receive prehospital crystalloid infusion or blood component transfusion resuscitation per site standard care for the respective EMS unit/service once all inclusion criteria and no exclusion criteria are met. Once the patient arrives at a participating site and prehospital initiated standard care transfusions are completed, patients will receive standard in-hospital trauma care.

Randomization and Masking: A single stage cluster randomization scheme will be utilized. Participating EMS services/agencies at each site will follow a fixed randomization approach of (LTWB O), (LTWB O), (Standard Care), with the initial treatment being randomly assigned. Thus an EMS service/agency will be randomly assigned to one of three three-month repeated

sequences: (1) LTWB O), (LTWB O), (Standard Care); (2) LTWB O), (Standard Care), (LTWB O); and (3) (Standard Care), LTWB O), (LTWB O)

Communication with the blood bank transportation services and each respective air base or EMS unit/service will occur and an annual schedule of randomization assignment will be distributed to each study site and respective EMS base/service. This specific randomization scheme is required due to the limited supply, goal of minimization of wastage of LTWB O, and the logistics and delivery of the intervention in the prehospital setting. The proposed trial will be an open label trial as blood product transfusion requires appropriate documentation and essential look back procedures.

III. Outcomes

Primary Outcome**:** The primary outcome for the clinical trial will be all cause 30-day mortality.

30-day mortality will be checked by calling the participant directly with the phone number they provided. If there is no response from the patient, 30-day mortality will be checked by searching public databases such as the National Death Index.

Secondary Outcomes**:** *Our principal secondary outcome for the trial will be age of whole blood and its association with all primary and additional secondary outcomes.* Additional secondary outcomes for the proposal will include 3-hour mortality, 6-hour mortality, 24-hour mortality, in hospital mortality, death from hemorrhage, death from brain injury, blood and blood component transfusion requirements in the initial 24 hours, incidence of Multiple Organ Failure (MOF), incidence of nosocomial infection, incidence of acute respiratory distress syndrome (ARDS), time to hemostasis, incidence of coagulopathy by TEG, incidence of allergic/transfusion reaction and measurements of platelet and overall patient hemostatic function.

Secondary Outcome Definitions:

Age of whole blood: Age of the LTWB O blood products transfused will be recorded as designated by the respective blood banking service at each respective site from the time of donation. Based upon the separation procedures utilized at the respective blood bank, LTWB O units will have a defined maximum shelf life out to 21 days or 35 days. Specific sites may have the capability to recycle LTWB O units to packed red blood cells and standard site-specific protocols will be utilized for this. When the age of 2 units of LTWB O are disparate, an average age of LTWB O transfusion will be calculated. We will stratify Age of LTWB O into YOUNG (1-14 days) and OLD (>14 days). We will similarly bin age or average age of LTWB O by week (1-7days), (8-14days), (15-21days), (22-28days), and > 28 days and allow comparison across all primary and secondary outcomes.

3-hour, 6-hour, 24-hour, and In-hospital mortality: 3-hour, 6-hour, 24-hour, and in hospital mortality will be recorded from the time of randomization. Over the first

24 hours, we will document and record the time of death in hours, while after the 24-hour time period, we will document and record the time of death in days from arrival. We suspect that patients in hemorrhagic shock will have a significant percentage of mortality occurring in the first 24-hour period.

Time to death: Time to death will be based on the documented and recorded time of death in days from arrival. Those surviving up to 30-days post enrollment will be censored.

Mortality from hemorrhage and brain injury: Cause of death will be adjudicated by a site investigator

Twenty four-hour blood and blood component transfusion requirements: 24-hour blood and blood component transfusion requirements will be determined by recording the type of product, the number of units transfused, and the time of transfusion. Any initiation of blood transfusion will be considered completed.

Multiple Organ Failure (MOF): Organ dysfunction will be evaluated via the Denver Post-injury Multiple Organ Failure Score, a well-validated scoring system in injured patients and characterized as an incidence rate (%) and as MOF free days.[14,15] Patients who are never admitted to the ICU or those with a length of ICU stay of less than 48 hours will be considered to have a Denver score of 0. A summary of the Denver score may be calculated by summing the worst scores of each of the individual systems. A summary Denver score > 3 will be classified as MOF. Scores will be determined daily up until Day 7 or ICU discharge, whichever comes first.[15]

Nosocomial Infection (NI): The CDC criteria for the diagnosis of hospital acquired pneumonia and blood stream infection will be utilized (www.cdc.gov/nhsn) and recorded over 30 days.

Acute Respiratory Distress Syndrome (ARDS): The Berlin definition for mild ARDS (PaO_2/FIO_2, ≤ 300 mm Hg + timing, imaging and origin criteria) will be utilized as a threshold value to determine the incidence of ARDS and will be further stratified into Moderate (PaO_2/FIO_2, ≤ 200 mm Hg) and Severe (PaO_2/FIO_2, ≤ 100 mm Hg).[16]

Coagulation parameters: During the first 60 minutes from arrival to the TOWAR trial site, blood for PT, INR and point of care rapid-TEG or conventional TEG analysis will be obtained. These measurements will be repeated as close to 24 hours (+/- 12 hours) from the time of arrival as feasible, to coordinate with other lab draws and staffing patterns. For those sites with rapid TEG capabilities, tissue factor will be added to a citrated whole blood collection tube and rapid TEG parameters including activated clotting time (ACT, seconds or R time), angle (α, degrees), coagulation time (K, seconds), maximum amplitude (MA, mm), clot strength (G, dynes/cm^2), and estimated percent lysis (LY30, %) will be measured for each patient.

Time to hemostasis: The time to hemostasis outcome variable will be determined by the ability to reach a nadir transfusion requirement of 1 unit of red blood cells in a 60-minute time period in the first 4 hours following arrival to the TOWAR trial site. Surgeon directed time to hemostasis will also be collected during the case when feasible. In the absence of the ability to obtain hemostasis by either of these criteria within the first 4 hours, the patient will be designated a 'non-hemostasis' patient.

Allergic/Transfusion reaction: Any transfusion complication in the prehospital setting will be monitored. As the intervention is specific to the early phase of care setting and since transfusion complications are temporally related to the specific transfusion, all transfusion related complications will be assessed during the initial 24 hours from the time of administration and recorded.

Platelet Function: We will assess platelet activation, aggregation, and adhesion to monitor the changes in platelet activity in recipients of whole blood of various ages of storage at sites with appropriate capabilities. Blood will be collected within 60 minutes of arrival to the TOWAR site when feasible and analyzed for aggregation via whole blood aggregometry using low dose collagen (2ug) as a stimulus. In addition, all patients will receive TEG (6s or 5000, depending on site availability) with platelet mapping utilizing ADP and arachidonic acid (AA) stimulation. Additionally, a rTEG will be run and the MA recorded. Collectively, the TEG with platelet mapping and the MA from rTEG will serve as quantitative assessment of clot strength, predominantly driven by platelet function. In a subset of patients presenting at centers with appropriate analytical capabilities, we will assess platelet activation via flow cytometry analysis for P-selectin expression (CD62p) as well as perform flow based microfluidic assays across collagen impregnated slides to assess for platelet adhesion under flow based conditions. Finally, due to the evolving importance of platelet extracellular vesicles in hemostasis and inflammation, when feasible we will perform size exclusion chromatography to isolate and characterize EVs from platelets collected from patients and utilize nanoparticle tracking analysis (Nanosight) to quantify the presence of EVs. [17,18]

Classification of Mortality**:** Classification of the underlying mechanisms responsible for mortality are essential to appropriately characterize regional variation and preventable morbidity and mortality. Classification of mortality outcomes will be assigned at the level of the enrolling institution by the respective Site Investigator. A predefined list of mortality classifications will be provided and adjudicated upon at the site level and will include
1) Hemorrhage/Exsanguination, 2) TBI/herniation, 3) Multisystem Organ Failure, 4) Sepsis, 5) ARDS, 6) Coagulopathy, 7) Cardiac Arrest with 1-6, 8) Pulmonary Embolism, 9) Withdrawal of Care as well as other pertinent causes of injury related death.

Predefined Subgroups**:** Predefined subset analyses for the trial will be performed looking at 1) Patients who ultimately did or did not required in-hospital blood transfusion; 2) Patients with and without significant traumatic brain injury (Head abbreviated injury scale- AIS >2 excluding cervical spine injury without TBI); 3) Patients arrived from the scene of injury versus those brought from a referral hospital; 4) Patients who ultimately did or did not require massive transfusion (≥ 10 units blood in first 24 hours); 5) Patients who received leukocyte reduced

whole blood versus those who did not; 6) Patients who had severe prehospital hypotension (SBP <70mmHg) versus those who did not; 7) Patients who required operative intervention in first 24 hours versus those who did not; 8) age, 9) sex; 10) race; 11) trauma center; and 12) service. It is recognized that the study is not appropriately powered for these subgroup comparisons and the results and conclusions formulated from these subgroup analyses will be considered exploratory in nature and will not be used as a basis for treatment recommendations. Please note that some of the planned subgroup analyses involve post-randomization factors. In these instances, mediation analyses will be carried out.

IV. Screening and Enrollment

Inclusion and exclusion criteria will be assessed based on available information at the time of enrollment in the prehospital environment. Although all reasonable efforts will be made by the EMS crews to either directly witness or obtain documentation of eligibility criteria, due to the nature of the emergency pre-hospital setting, there may be occasions where the EMS crew must rely on verbal report of inclusion criteria, including qualifying vitals, from the referring hospital or ground crew. In these instances, if, after subsequent review of outside hospital and/or ground crew documentation, it is determined that the subject did not meet inclusion criteria and/or met exclusion criteria, the subject will remain enrolled in the study based on the intention-to-treat principle.

In the event that a verbal report must be used in lieu of physical documentation or directly witnessing the qualifying vitals, documentation of the verbal report will serve as the source documentation for determining eligibility. Verbal reports will be documented in the emergency medical record and will detail the information reported and by whom.

Study participants will be randomly assigned to either LTWB or standard of care based on a cluster randomized design. Patients will be randomly assigned by EMS base to receive either standard of care (one-month time interval) or LTWB (two-month time interval).

Subjects will undergo initial blood sampling for our secondary outcomes of interest during the first 60 minutes from arrival to the TOWAR trial site. A point of care rapid or conventional thromboelastography (TEG) will be performed for coagulation parameter measurements as close to arrival as possible and as close to 24 hours as possible (+/- 12 hours) when clinical care allows.

V. Potential Risks and Benefits

Potential Risks

Risks Associated with LTWB: The whole blood product to be utilized starting in the prehospital environment is similar to the current whole blood product that has been transfused in 939 patients over the last 45 months at the University of Pittsburgh without evidence of complications or transfusion reactions. There have been no hemolytic transfusion reactions in patients transfused LTWB at the University of Pittsburgh in the last 45 months. The primary risks with any blood transfusion can be found in the Circular of Information prepared jointly by AABB, the American Red Cross, America's Blood Centers, and the Armed Services Blood Program. Those primary acute immunologic complications include hemolytic transfusion

reactions, immune mediated platelet destruction, febrile nonhemolytic reactions, allergic reactions, anaphylactoid reactions and transfusion-related acute lung injury (TRALI). Delayed immunologic complications include delayed hemolytic reaction, alloimmunization, posttransfusion purpura, and transfusion-associated graft-vs-host disease. Nonimmunologic complications include transmission of infectious agents, bacterial sepsis, transfusion-associated circulatory overload (TACO), hypothermia, and metabolic complications.

The intervention product is group O LTWB. A risk associated with transfusion of Rh+ blood in a woman of child-bearing age is hemolytic disease of the fetus during a future pregnancy. Complications of this disease include fetal anemia, fetal hydrops, and in the most severe cases, fetal death. However, the overall risk of poor fetal outcome in a future pregnancy for Rh- women that are administered Rh+ blood has been estimated to be less than 0.5%.[19] Importantly, the incidence of a female being Rh- is 10-15%. The majority of women injured have no risk of Rh mismatch. For those that are at risk, the likelihood of poor fetal outcome in a future pregnancy is small. Importantly, an FDA approved EFIC pilot, prehospital thru in-hospital, LTWB randomized trial (PPOWER IND #17736) randomized group O RH+ LTWB and included women of childbearing age due to this low inherent risk. Women of childbearing potential who receive O+ whole blood resuscitation and are determined to be Rh- will be notified and counseled following standard blood banking and trauma service guidelines.

Potential Benefits/Safety

The University of Pittsburgh has over the last 4 years initiated an urgent release whole blood transfusion program for trauma patients in hemorrhagic shock in our emergency department utilizing LTWB identical to the proposed intervention large multicenter trial. We published data on 172 patients demonstrating the feasibility and safety of such a program.[20,21] We have continued this program and have currently utilized 2176 units of LTWB in over 939 patients with no safety concerns. We have simultaneously been monitoring LTWB patients for hemolytic side effects from ABO mismatched whole blood. In 102 non-group O patients, there were no significant differences in laboratory hemolysis markers.[22] We have also demonstrated that LTWB is superior in its hemostatic potential, its ability to reverse shock and its association with reduced transfusion volumes when administered in children.[23] These published data verify the feasibility, safety and potential benefits of this type of intervention in patients with hemorrhagic shock, providing the basis for the successful execution of the proposed multicenter trial. We have also executed a pilot prehospital thru in-hospital, LTWB randomized trial (PPOWER IND #17736) with one of the primary outcomes being feasibility. The experience from this NIH funded pilot trial have been leveraged for the current prehospital multi-center trial.

VI. Statistical Analysis Plan

Analysis for Trial Clinical Outcomes:

AIM#1 Analysis: Determine whether prehospital low titer whole blood as compared to standard prehospital resuscitation results in lower 30-day mortality in patients at risk of hemorrhagic shock.

The analysis will begin by describing the baseline demographic and clinical characteristics of the overall population and then stratified by treatment arm to compare those who receive LTWB and those who receive standard care. For discrete variables, proportions will be generated, and a chi-square test will be used to test for differences between the proportions. For continuous characteristics, means (medians) and standard deviations (interquartile ranges) will be calculated and t-tests (Wilcoxon) will be used to compare the means (distributions) between treatment arms.

For the primary trial outcome, an indicator of death within 30 days will be generated for each participant. A two-sided z-test for proportions controlling for within cluster correlation utilizing the methods of Donner and Klar will serve as the primary approach to compare the proportions between the treatment arms.[24] A mixed-effects logistics regression model will be used to assess the independent impact of LTWB on 30-day survival after controlling for potential confounding effects of baseline characteristics which reveal imbalance between treatment groups. Random effects will be included for intercept, slope, service. Fixed effects will include treatment, baseline characteristics not balanced through random assignment, and month of study.

Analyses to test for the homogeneity of the treatment effect will be carried out for the pre-defined subgroups. Regression models appropriate for the outcome variable (e.g., mixed effect logistic regression for binary outcome variables) will be used to test for the homogeneity of the treatment effect. Main effects will be included in the model for treatment, the indicator of the subgroup and the two-way interaction between treatment and subgroup. If a statistically significant interaction is observed, we will reject the null hypothesis of a homogenous treatment effect.

AIM#2 Analysis: Determine whether prehospital old whole blood (age > 14 days) as compared to young whole blood (age ≤ 14 days) is associated with similar clinical outcomes, hemostasis, prevention of coagulopathy, and platelet function in patients at risk of hemorrhagic shock.
The analysis will begin by describing the baseline demographic and clinical characteristics of the overall population and then stratified by age of whole blood; whole blood age ≤ 14 days versus whole blood age > 14 days. For discrete variables, proportions will be generated, and a chi-square test will be used to test for differences between the proportions. For continuous characteristics, means (medians) and standard deviations (interquartile ranges) will be calculated and t-tests (Wilcoxon) will be used to compare the means (distributions) between treatment arms.

An unadjusted analysis comparing the 30-day mortality rate by blood age will be conducted using a chi-square test. Additionally, because of the observational nature of the study, a propensity adjusted analysis will be carried out to control for potential confounders (including biological sex, TBI, etc.). A generalized boosted regression model (GBM) will be used to generate a propensity score for each participant indicating the probability of receiving young whole blood. The GBM approach will be used to identify important characteristics that will be used to estimate the propensity scores. From this approach, a propensity score, $p(x)$, indicating the probability of receiving young whole blood given the observed characteristics will be obtained for each subject.

We will use the Toolkit for Weighting and Analysis of Nonequivalent Groups (twang) software package and the SAS/Stata macros (http://www.rand.org/statistics/twang/downloads.html) to estimate and evaluate the propensity scores. We will estimate the propensity score for each

subject by running the macro or command *ps* (dichotomous) or *mnps* (multinomial). That is, GBM will fit separate GBMs to each dummy treatment indicator to estimate the propensity score for the given phenotype. We will consider all the subject characteristics when fitting GBM. The GBM approach will select which characteristics to include and the best functional form including interactions. If missing data is present for the subject characteristics, twang will attempt to construct weights that also balance rates of missingness by creating indicator variables.

The maximum number of iterations for the GBM approach will be set to the default (i.e.10000). The optimal number of iterations will be selected as the one that minimizes the balance statistics of interest. We will use the balance statistics es.mean and ks.mean. The es.mean uses the absolute standardized bias (also referred as the effect size or absolute standardized mean difference) and summarizes across variables, and the ks.mean uses the KS statistic to assess balances and summarizes across variables. We will allow a maximum of three splits for each tree in the model, allowing for three-way interactions between all covariates to be considered. The shrinkage parameter will be set to 0.0005 to ensure a smooth fit. The tuning parameters involved in the GBM models will be adjusted if necessary, until covariate balance is achieved.

To evaluate the model, we will plot the balance statistics as a function of the number of iterations (higher iterations correspond to more complicated fitted models). If more iterations are needed, we will increase the number of iterations. Balance tables and plots will be generated to assess balance. Multiple pairwise sets of balance metrics will be evaluated. We will use the ES and the KS as balance metrics to assess whether the groups are balanced with respect to the observed baseline and pre-treatment characteristics. For the ES metric, a value under 0.20 is indicative of good balance. For the KS metric, a value under 0.10 will be indicative of good balance, but p-values will also be evaluated. The GBM fit (es.mean or ks.mean) that provides the best balance and yields the largest effective sample size will be preferred (McCaffrey et al., 2013). We will also plot the propensity score values using boxplots to compare the distributions and to evaluate the common support. Ideally, the propensity score distributions will overlap entirely; however, if there are areas of substantial non-overlap, we will discard subjects in those areas to increase generalizability. We will use a 1-dimensional function of the propensity score, referred to as the sensitivity function (SF), to quantify the hidden bias due to unmeasured confounders.

Variables in the GBM will include demographic characteristics, clinical characteristics, and site. A mixed-effects log-binomial regression model will then be used to estimate the independent effect of receiving young whole blood on 30-day mortality. The mixed effects log-binomial regression model will include an indicator of 30-day mortality as the dependent variable and indicator of the age of whole blood as the independent variable, with the propensity scores used as an inverse probability weight.

A similar approach will be taken for the secondary outcomes, though the analytic approach may vary based on the distribution of the outcome. For example, for a continuous secondary outcome (e.g., clotting time parameters); a mixed-effects linear regression model to estimate the independent effect of the age of whole blood on the outcome. For time to event outcomes (e.g., time to death), Kaplan-Meier curves will be generated for each treatment group and a log-rank test will be used to compare the distribution of the cumulative proportion. Observations will be censored at 30 days after arrival. A multivariable analysis of survival with the use of a Cox

proportional-hazard with shared frailty model to evaluate the treatment effect with adjustment for possible confounding factors and site clustering on survival.

AIM#3 Analysis: Determine whether prehospital low titer whole blood as compared to standard prehospital resuscitation results in lower early mortality endpoints, blood and blood component transfusion requirements, lower incidence of coagulopathy, and improved hemostatic and platelet function in patients at risk of hemorrhagic shock.

The analysis of the secondary outcome will mirror the analytic approach for Aim 2. The analytic approach for other binary outcomes (e.g., 6-hour mortality) will be identical as those for the primary analysis. For continuous outcomes (e.g., 24-hour blood and component transfusion requirements), a mixed effect regression model will be used to determine if the average number of units transfused between the two treatment groups differ. For time to event outcomes (e.g., time to hemostasis), Kaplan-Meier curves will be generated for each treatment group and a log-rank test will be used to compare the distribution of the cumulative proportion. A multivariable analysis of survival with the use of a Cox proportional-hazard with shared frailty model to evaluate the treatment effect with adjustment for possible confounding factors and site clustering on survival.

Sample Size Justification and Power Analysis: The sample size calculations are based on the primary outcome, 30-day mortality, for Aim 1 of the study. With 40 service providers, a sample of 340 participants per treatment group is needed to detect a difference in the 30-day mortality rate of 26% vs. 16% (based on data from the PAMPer study), assuming 80% power, a type I error rate of 0.05, a two-sided alternative hypothesis, and an intraclass correlation coefficient of 0.02, as was observed in the PAMPer study.[12] The size of the ICC will have an impact on the size of the sample needed. If the ICC is smaller (e.g., 0.01 would require a sample size of 300 per arm), fewer people will be needed. However, if it is larger, more will be needed (e.g., 0.05 would require a sample size of 680 per arm).

Using a 2:1 random allocation of study participants to LTWB:standard of care, and enduring that there are 340 participants in the standard care arm, will lead to a total sample size of 1020, with 680 participants receiving LTWB. Assuming that 60% of these participants will receive whole blood ≤ 14 days in age and 40% will receive whole blood > 14 days in age, based on a chi-square test of proportions there will be 80% power to detect a higher mortality rate of 22.6% among those receiving whole blood over 14 days old, assuming a 30-day mortality rate of 14% among those receiving whole blood ≤ 14 days, a type 1 error of 0.05 and a two-sided alternative hypothesis (that whole blood > 14 days in age has a worse outcome than whole blood ≤ 14 days) vs. the null hypothesis that whole blood > 14 days is the same or better than whole blood ≤ 14 days).

Randomization of Ineligible Subjects: It is anticipated that there will be a small proportion of patients enrolled who receive whole blood or standard care that in retrospect will not have met the entry criteria and are thus ineligible. In this circumstance, patients will be analyzed according to the group to which they were randomized. Subgroup analyses based on eligibility criteria will be performed if the number of patients so affected is large. However, based on the relatively

limited inclusion and exclusion criteria it is anticipated that the frequency of this event will be low.

Missing Data: Consistent with the intention-to-treat principle, the analysis methods used to analyze the primary outcome include all patients according to their assigned intervention. Baseline characteristics of patients with missing outcome data will be compared. Missing primary outcome data will be imputed. Based on data from PAMPer, it is anticipated that the missing data rate will be less than 5%.[12]

Non-adherence: In some circumstances, patients may receive standard care instead of the whole blood intervention when randomized to whole blood. Non-adherence is most likely to occur in the case of the patient who requires urgent care in the prehospital environment where logistics do not allow the intervention to be administered. Fortunately, this event is relatively rare. In keeping with the intention-to-treat analytic design, these patients will be analyzed with the group to which they were randomized.

Interim Analyses**:** In concert with the DSMB, prior to initiation of the trial, the final monitoring plan will be developed to serve as the guide to the DSMB's decision-making process concerning early stopping of the trial. In making the decision to recommend termination of the study, the DSMB shall be guided by several types of information: (i) a formal stopping rule based on the primary analysis, (ii) information on safety outcomes by treatment group, (iii) consistency between results for primary and secondary outcomes, and (iv) consistency of treatment effects across subgroups.

We have designed this trial with two-interim looks before the final analysis. The groups sequential approach of Lan and DeMets using the O'Brien-Fleming spending function will be used to determine the type I error boundaries for each analysis. The selection of the Lan and DeMets approach permits flexibility with respect to the number of interim analyses. Two formal interim analyses of efficacy will be performed when 33% and 67% of the enrolled participants reach the assessment of the primary endpoint (30 days after injury). The level of significance will maintain an overall p value of 0.05 according to stopping boundaries leaving a p value of 0.038; two sided, for the final analysis with a final z-value of 1.993.

The DSMB may recommend termination of the trial if the results of the interim analysis are unlikely to change after accruing more patients based on conditional power. Conditional power is defined as the approach that quantifies the statistical power to yield an answer different from that seen at the interim analysis. If this quantity is small, then the DSMB may conclude that it would be futile to continue with the investigation.

Data Sources**:** Data will be collected prospectively as patient care progresses. This will include a review of the prehospital and emergency medical patient care report(s), Emergency Department and electronic/paper hospital records.

Data Entry**:** The DCC will create web-based HTML forms to collect necessary information from all participating sites. Web entry forms will have dynamic features such as edit and data type checks. Details and clarification about data items will be provided using pop-up windows. Data

encryption and authentication methods will be used. Additional features will be built into the web entry forms including: forms transmission history, access to past forms, tracking of data corrections, and the capability to save and re-load incomplete forms. The subjects will be identified by a study number only. All clinical interventions will become part of the patient's medical records including platelet transfusion. All hard copy source documentation will be kept in a secured, locked cabinet in the site's research coordinator's office. All study documents will be maintained in a secure location for the time frame designated by each participating site's requirements. The electronic data will be entered and maintained on a password protected SSL website designed for this trial.

The data entered for the TOWAR trial will be maintained by the DCC on a relational database. The database would be housed in a virtual environment so in the event of a hardware failure it would migrate to a new host. The data will be backed on a regular schedule with full transaction log files in use and copies of the data will be stored offsite with a secure service. In addition to the data server, the production web server will also be backed up routinely and as a virtual machine can be transitioned to different hardware automatically in case of hardware failure. All Servers are behind an enterprise firewall and access has to be granted through the firewall even within the University Network.

Database Management**:** A two-tiered database structure will be created. A front-end database will serve the web entry needs, using a database management system well-suited to handling updates from multiple interactive users. The data from this database will be transferred on a regular schedule to a data repository that can be used by statistical software packages. This database will be kept as needed, e.g. for quarterly performance reports. Access to data will be limited to those who need access to perform their tasks. The database management system is able to manage large quantities of data, to merge data from multiple databases as required, to handle complex and possibly changing relationships, and to produce analysis datasets that can be imported into a variety of statistical analysis packages.

Surveillance for Outcomes and Data Elements**:** Data will be collected prospectively as patient care progresses. This will include a review of prehospital and emergency medical patient care report(s), Emergency Department and electronic/paper hospital records.

> Prehospital and In-Hospital Data: Demographics, shock severity (base deficit, lactate), injury characteristics, Prehospital and ED vitals, interventions (intubation, chest tubes), injury severity, operative interventions and timing of interventions, injury severity score, ICU days, ventilator days, length of stay, multiple organ dysfunction scores (daily), nosocomial infectious outcomes, blood gas results, radiography reads, transfusion of blood and blood components, resuscitation requirements, all primary and secondary outcomes will be recorded.

VII. Clinical Coordinating Center (CCC)

Clinical Coordination specific for the TOWAR study will be performed by MACRO (Multidisciplinary Acute Care Research Organization) and the dedicated research teams at the

University of Pittsburgh, including all regulatory requirements, provider and coordinator training and monitoring.

VIII. Data Coordinating Center (DCC)

Data Coordination specific for the TOWAR study will be performed by the DCC and led by Dr. Wisniewski at the Graduate School of Public Health at the University of Pittsburgh. The DCC will coordinate all data collection and entry, management, security and confidentiality, data archiving, quality control and electronic medical record biomedical informatics as needed, as well as plan, coordinate and assist with all statistical analyses

IX. Human Subjects

We anticipate that this study will be conducted under the federal provisions governing Exception from the Requirement for Informed Consent for Emergency Research, including community consultation, public notification, as well as notification of patients or their legally-authorized representative as soon as feasible after enrollment. The latter shall include provision of an opportunity to opt out from ongoing participation that will be given through oral and written communication.

Community consultation as determined by the central IRB will be undertaken prior to final IRB approval. We will utilize a central IRB at the University of Pittsburgh. Since the population eligible for enrollment includes all citizens in the study regions it will not be possible to target any particular small group. Feedback from the community will be obtained by research personnel regarding any concerns they may have about potential enrollment. If requested, bracelets will be made available that could be worn by members of the community who do not want to participate. Public notification and community consultation will be performed as directed by the central IRB and may include such methods as surveys of the proposed study community, targeted small group meetings or consultation with community leaders. Due to ongoing participation in numerous multicenter research studies involving emergency research, our institution and the other participating institutions have significant experience with community consultation and notification practices.

Institutional Review Board: A central IRB will be utilized at the University of Pittsburgh for the regulatory needs of studies. All current LITES Network sites have IRBs which have experience and engagement with central IRB procedures.

Training and Participating Site Coordination**:** As the clinical coordinating center for the trial, the University of Pittsburgh (MACRO) at the University of Pittsburgh will be collaboratively responsible for all research coordinator training, provider training and sample collection and storage. Research coordinators, providers and associated staff will be trained during the months prior to the trial start date regarding the scientific basis for the study, specific inclusion and exclusion criteria, sample collection and processing, study procedures and SOPs, and rapid TEG performance. Training verification and retraining will occur if new staff is hired at individual participating sites. Trial enrollment and maintenance of data integrity will be assessed using the

web-based data platform. Trial screening, enrollment and data completeness and accuracy will be assessed at predefined time periods via site visit and random patient audit.

X. Safety Monitoring

Adverse Event and Non-compliance Definitions

a. Adverse event means any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related.
b. Adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused the event.
c. Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction"
d. Reasonable possibility. For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.
e. Unexpected adverse event/reaction refers to an event/reaction that is not consistent with the risk information described in the general investigational plan or elsewhere in the IND application.
f. Life-threatening, suspected adverse reaction. A suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or research subject at immediate risk of death. It does not include a suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
f. Serious, suspected adverse reaction. A suspected adverse reaction is considered "serious" if, in the view of the Investigator (i.e., the study site principal investigator) or Sponsor, it results in any of the following outcomes: death, a life-threatening adverse reaction, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.
g. *Reportable non-compliance* refers to a failure on the part of the investigator or study team member to follow the terms of the IRB approved protocol or abide by applicable laws or regulations, that adversely affect the rights and welfare of subjects or significantly compromises the quality of the research data. Incidents of non-compliance on the part of the subject are not considered reportable.
h. Unanticipated Problem Involving Risk to Subjects or Others (UPIRTSO) refers to any accident, experience, or outcome that meets the following criteria: unexpected in terms of nature, severity or frequency; related, or possibly related, to a subject's participation in research; and places subjects or others at greater risk of harm (including physical, economic, or social) than was previously known or recognized.

Assessing and Reporting Adverse Events (AEs) and Non-compliance: Adverse events will be reviewed by the study sites and assessed for relationship to the study intervention. Investigators and study team will determine if any related adverse events occur during the period from

enrollment through study participation termination. If reportable adverse events occur, they will be recorded on the adverse event case report form in the electronic data capture system, which will be submitted to the Coordinating Center. All reported adverse events will be classified by: a) Severity (fatal or life-threatening, serious, or non-serious); and b) Expected vs. Unexpected. Please refer to the table below for timelines for reporting.

This study population is expected to have a large number of serious adverse events, including death from trauma related injuries. Expected adverse events that are related or possibly related to the intervention will be documented and reviewed for changes in nature, severity, or frequency across the study population.

Organization	Unexpected, fatal or life-threatening, suspected adverse reactions	Unexpected, serious, suspected adverse reactions	Expected adverse reactions	Reportable non-compliance	UPIRTSO
IRB	24 hours	10 working days	No reporting	10 working days	10 working days
FDA	7 calendar days	15 calendar days	No reporting	No requirement	No requirement
Dept of Defense	30 calendar days	30 calendar days	No reporting	30 calendar days**	30 calendar days*
DSMB	24 hours	7 calendar days	At next meeting (every 6 months)	At next meeting (every 6 months)	14 days*

*reported based on IRB determination that event is UPIRTSO
**reported based on IRB determination that non-compliance is serious or continuing

Data Safety Monitoring Board (DSMB)**:** A Data and Safety Monitoring Board (DSMB) will be created to review this study and provide recommendations re. study continuation to the IND Sponsor. After initial approval and at periodic intervals (to be determined by the committee) during the course of the study, the DSMB responsibilities are to:

a. Review the research protocol, informed consent documents and plans for data and safety monitoring;
b. Evaluate the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, adverse events, unanticipated problems, performance of the trial sites, and other factors that can affect study outcome;
c. Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the

participants or the ethics of the study;
d. Review clinical center performance, make recommendations and assist in the resolution of problems reported by the IND Sponsor or study site Investigators;
e. Protect the safety of the study participants;
f. Report on the safety and progress of the study;
g. Make recommendations to the IND Sponsor, and if required, to the FDA concerning continuation, termination or other modifications of the study based on the observed beneficial or adverse effects of the treatment under study;
h. Monitor the confidentiality of the study data and the results of monitoring;
i. Assist the IND Sponsor by commenting on any problems with study conduct, enrollment, sample size and/or data collection.
j. The DSMB will include experts in emergency medicine, surgery (trauma/critical medicine), bioethics and biostatistics. Members will consist of persons independent of the investigators who have no financial, scientific, or other conflict of interest with the study. Written documentation attesting to absence of conflict of interest will be required.
k. The University of Pittsburgh Office of Clinical Research, Health Sciences will provide the logistical management and support of the DSMB. A safety officer (chairperson) will be identified at the first meeting. This person will be the contact person for serious adverse event reporting. Procedures for this will be discussed at the first meeting.
l. The first meeting will take place before initiation of the study to discuss the protocol, approve the commencement of the study, and to establish guidelines to monitor the study. The follow-up meeting frequency of the DSMB will be determined during the first meeting. An emergency meeting of the DSMB will be called at any time by the Chairperson should questions of patient safety arise.

XI. Quality Control, Assurance and Confidentiality

Protocol Compliance: The participating study site Investigators will not deviate from the protocol for any reason without prior written approval from the IRB except in the event of the safety of the research subject. In that event, the study site Investigator will notify the IND Sponsor and reviewing IRB immediately, if possible, and request approval of the protocol deviation, or, if prospective IND Sponsor and IRB approval is not possible, the study site Investigator will notify the IND Sponsor and reviewing IRB promptly following the respective protocol deviation. The study site Investigator will inform the reviewing IRB of all protocol deviations and unanticipated events involving risks to the research subjects and others and will obtain prospective IRB approval for all proposed protocol changes. Persistent or serious noncompliance may result in termination of the study site's participation in the research study.

Protocol Deviations: Due to the relative focused inclusion criteria and the short intervention period, we expect few protocol deviations as compared to other large multicenter trials. If monitoring reports demonstrate evidence of continuing protocol deviations, we will analyze them to determine if they are site specific or common across the study. We will note if specific inclusion or exclusion criteria are being misinterpreted, if a certain time point in testing is being omitted, or if a common set of data elements are missing. If the deviations are site specific, retraining will be done at the site. If the problems are study wide, we will discuss them with the

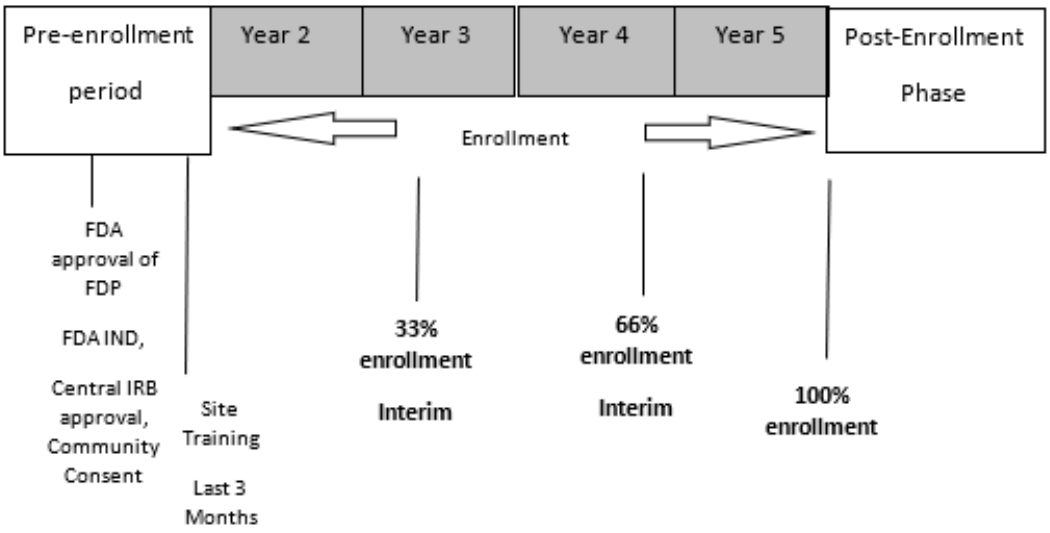
other investigators, the DOD and the FDA to see if the protocol needs to be amended or recruitment put on hold.

Privacy and Confidentiality: The study site Investigators and members of their research team will make reasonable effort to ensure the research subjects' confidentiality. Subject name and other identifiable information will be kept in a secure, locked, limited access area.

Investigator Responsibilities:

The study site Investigators will agree to implement the IRB approved protocol and conduct the study in accordance with Section 9 (Commitments) of Form FDA 1572, 21 CFR Part 312, Subpart D, and the ICH GCP Guidelines (E6, Section 5) as well as all applicable national, state and local laws. The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, and applicable regulatory requirements.

Timetable



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Appendix I Requirements for Exception From Consent For Emergency Research

We have outlined below each criterion stipulated in the regulations for this exception and how our study design applies to these criteria.

(1) The human subjects are in a life-threatening situation, available treatments are unproven or unsatisfactory, and the collection of valid scientific evidence, which may include evidence obtained through randomized placebo-controlled investigations, is necessary to determine the safety and effectiveness of particular interventions.

The proposed trial is a randomized trial comparing the use of prehospital LTWB versus standard of care in patients in hemorrhagic shock following injury. These patients are in a life-threatening situation with a mortality approaching 30-40% despite all efforts.[12] The standard of care for management of these patients generally includes crystalloid resuscitation and in some instances red cell transfusion in the prehospital environment on air medical and rare ground EMS services with blood transfusion capabilities. Importantly, prior studies have demonstrated that injured patients who require large volume blood transfusion have improved survival if transfusion of high or equal ratios of plasma and platelets to blood occurs.[3] Evidence suggests that early blood component transfusion may reduce overall blood transfusion requirements and that addressing the coagulopathy which occurs early after injury improves outcome.[1-3,12,25] This is what whole blood resuscitation provides.

(2) Obtaining informed consent is not feasible because:
i. The subjects will not be able to give their informed consent as a result of their medical condition;
ii. The intervention under investigation must be administered before consent from the subjects' legally authorized representatives is feasible; and
iii. There is no reasonable way to identify prospectively the individuals likely to become eligible for participation in the clinical investigation.

The study intervention needs to be administered en-route to a definitive trauma center from the injury scene or outside emergency department to provide the maximal survival benefit.[12] (see discussion of therapeutic window below). In this uncontrolled setting, the hemorrhagic shock patient is unable to provide consent for study enrollment, as they are commonly unconscious or in extremis, and legal next-of-kin are often not immediately available, nor is it practical for the prehospital provider to explain the study and receive consent while caring for the patient emergently. Since we are studying patients with hemorrhagic shock following injury, there is no way to prospectively identify individuals who are likely to become eligible for this trial.

(3) Participation in the research holds out the prospect of direct benefit to the subjects because:
i. Subjects are facing a life-threatening situation that necessitates intervention;

ii. Appropriate animal and other preclinical studies have been conducted, and the information derived from those studies and related evidence support the potential for the intervention to provide a direct benefit to the individual subjects; and
iii. Risks associated with the investigation are reasonable in relation to what is known about the medical condition of the potential class of subjects, the risks and benefits of standard therapy, if any, and what is known about the risks and benefits of the proposed intervention or activity.

(i) As defined, these patients are injured and in hemorrhagic shock and are facing a potentially life-threatening situation that requires immediate intervention.
(ii) Previous human studies suggest the potential for a direct benefit to individual patients who are in hemorrhagic shock.[3,9,10,12,26]
(iii) Whole blood has been evaluated in the military and has been shown to offer a survival advantage. We have provided whole blood to over 939 patients without complication or incident. As discussed above, there are potential risks to subjects that may have not been observed in previous trials. We contend that these risks are reasonable in light of the potential benefits outlined in this proposal and the current poor outcome for patients with hemorrhagic shock.

(4) The clinical investigation could not practicably be carried out without the waiver.

This study could not be conducted without the waiver of consent due to the need to initiate the intervention quickly in the prehospital setting en route, prior to trauma center arrival for hemorrhagic shock patients at significant risk of mortality.

(5) The proposed investigational plan defines the length of the potential therapeutic window based on scientific evidence, and the investigator has committed to attempting to contact a legally authorized representative for each subject within that window of time and, if feasible, to asking the legally authorized representative contacted for consent within that window rather than proceeding without consent. The investigator will summarize efforts made to contact legally authorized representatives and make this information available to the IRB at the time of continuing review.

Patients in hemorrhagic shock following injury have been shown to develop progressive hypothermia, coagulopathy and acidosis leading to further recalcitrant hemorrhage and multisystem organ failure and death.[2] There is no acceptable window of time to delay treatment in order to conduct an informed consent discussion with the subject. Since this is an immediately life-threatening situation, it will not always be possible to contact legal representatives at the time of study entry. We will make every effort to contact legal representatives as soon as feasible to notify them that the patient was enrolled in a randomized trial. Research personnel will attempt to contact the subject's legal authorized representative as soon as feasible and a summary of these efforts will be documented in the patient's chart. If the subject becomes competent during the study period, then he/she will be approached by research personnel for notification of enrollment.

(6) The IRB has reviewed and approved informed consent procedures and an informed consent document consistent with Sec. 50.25. These procedures and the informed consent document are to be used with subjects or their legally authorized representatives in situations where use of such procedures and documents is feasible. The IRB has reviewed and approved procedures and information to be used when providing an opportunity for a family member to object to a subject's participation in the clinical investigation consistent with paragraph (a)(7)(v) of this section.

All procedures and consent forms will be approved by the Single Institutional Review Board (sIRB) of the study prior to the onset of the trial.

(7) Additional protections of the rights and welfare of the subjects will be provided, including, at least:
i. Consultation (including, where appropriate, consultation carried out by the IRB) with representatives of the communities in which the clinical investigation will be conducted and from which the subjects will be drawn;
ii. Public disclosure to the communities in which the clinical investigation will be conducted and from which the subjects will be drawn, prior to initiation of the clinical investigation, of plans for the investigation and its risks and expected benefits;
iii. Public disclosure of sufficient information following completion of the clinical investigation to apprise the community and researchers of the study, including the demographic characteristics of the research population, and its results;
iv. Establishment of an independent data monitoring committee to exercise oversight of the clinical investigation; and
v. If obtaining informed consent is not feasible and a legally authorized representative is not reasonably available, the investigator has committed, if feasible, to attempting to contact within the therapeutic window the subject's family member who is not a legally authorized representative, and asking whether he or she objects to the subject's participation in the clinical investigation. The investigator will summarize efforts made to contact family members and make this information available to the IRB at the time of continuing review.

(i) Community consultation as outlined by the sIRB will be undertaken prior to IRB approval. Since the population eligible for enrollment includes all citizens in the study region it will not be possible to target any particular small group. Feedback from the community will be obtained by research personnel regarding any concerns they may have about potential enrollment. If requested, bracelets will be made available that could be worn by members of the community who do not want to participate. Public notification and community consultation will be performed as directed by the sIRB and may include such methods as using online surveys of the proposed study community, targeted small group meetings or consultation with community leaders if the pandemic allows it. Our institution has significant experience with community consultation and notification practices.

(ii) & (iii) Public disclosures will be performed both prior to study enrollment and at the completion of the study in the form of multimedia press releases organized by the investigators. These will include plans for the study including potential risks and benefits and a summary of the results of the study upon completion. In the event that the press releases are not widely circulated, advertisements may also be placed in local papers describing the study.

(iv) The Data Safety Monitoring Board will function as an independent data monitoring committee who will exercise oversight of the study. (v) We expect that all patients who meet the enrollment criteria will be unconscious or in critical state that does not allow appropriate consent to occur. Any delay in medical care that would be required for the care provider to attempt to obtain consent from the patient's legal guardian would be life threatening. Thus, it will not be feasible to attempt to obtain informed consent during the initial therapeutic window. Requiring consent to review a prehospital chart to determine the presence or absence of serious adverse events is likely to be associated with a biased estimate of the safety and efficacy of the intervention. Therefore, we will use exception from consent for emergency research which includes public notification, community consultation, patient notification of enrollment, and provision of an opportunity to opt out from ongoing participation.

Appendix II Donor -Patient LOOKBACK

DONOR-PATIENT "LOOKBACK"

APPROVALS

All Approvals are maintained and controlled via Document Control Systems' MC3 Portal™ Software. Please Refer to MC3 Portal™ for the current controlled revision and approval records.

SUMMARY OF THE MODIFICATIONS – See MASTERControl™ InfoCard Release Date

List a summary of the modifications below. Bullet outline is recommended.

Revised Principle: Regulatory agencies require notification of consignees by donor centers of blood products from a donor who subsequently tests confirmed positive for HIV1, 2, HCV, or HTLV-I/II or is at risk for transmitting Creutzfeldt-Jakob disease (CJD). Lookback will also be performed by CTS when notified by the blood center of donors confirmed positive for HBV, HTLV, WNV, Zika, and Ebola viruses and Babesia.

Revised Section II title and step II.A: RECIPIENTS PHYSICIAN NOTIFICATION OF POSSIBLE VIRAL/CJD/PARASITIC INFECTION (HIV, HCV, HBV, HTLV, WNV, Zika, Ebola, CJD, Babesia Ebola)

A. General Requirements: FDA has specific lookback requirements for some infectious agents, e.g., HIV, HCV, CJD, while others are performed by CTS as being important for patient safety (HVB, HTLV, WNV, Zika, Ebola, Babesia). The CTS Pittsburgh and Chicago physician will identify a transfusion recipient according to records available described in Section IV of this policy. Transfusion Service physician will send the patient's physician a letter or **secure e-mail** notifying him/her of the lookback. HIV and HCV notifications are sent by **certified mail or secure e-mail**. The rest of the notification letters are sent by regular mail or secure e-mail.

Revised step II.B.1; step II.B.5 rewritten

B.Lookback Notification Process

1.HIV and HCV lookback process: A notification letter is sent along with a notification form **by certified mail or secure e-mail**. The physician must promptly return the enclosed notification form

…

5.CJD lookback notification process and criteria: The blood center will notify CTS of components from donors that were found to have CJD, vCJD, suspected vCJD, risk factors for CJD, or if withdrawal is recommended in cases under investigation for vCJD (CJD diagnosis and age less than 55). In those situations, consignee notification could enable the consignee to inform the physician, or other qualified personnel responsible for care of the recipients, so that recipient tracing and medically appropriate notification and counseling may be performed at the discretion of health care providers. CTS will notify the patient's physician in writing by letter or secure email and request that he/she provides follow up to the transfusion service of the patient outcome. Follow up notification is not required if no response is received. If the patient is deceased, physician notification is not required.

For transfusible components from a donor with one family member diagnosed with CJD, or with risk factors for vCJD, suspected vCJD (due to geographic risk deferral, transfusion in the U.K. or in France between 1980 and the present, or due to injection of bovine insulin), per FDA Guidance for Industry, it is not appropriate to conduct tracing and notification of recipients of prior donations.

(Continued on next page)

Revised step V.A.4

V.RECORDS

A.The following Documents are maintained in the lookback file.

1. 4.Certified mail receipts **or print out of secure e-mail communication.**

D. Lookback records are maintained **for 10 years.**

E.HCV and HIV lookbacks are discussed at Transfusion Committee meetings and they are documented in Transfusion Committee records.

- **Deleted Procedure Notes 2-4**

PROCESS
SYSTEM
Investigation of Adverse Transfusion Effects, Information Management
CRITICAL CONTROL POINT
Documentation/Record Keeping, Supplier Qualification, Error/Accident Review, Internal Assessment, Process Improvement

PRINCIPLE
Regulatory agencies require notification of consignees by donor centers of blood products from a donor who subsequently tests confirmed positive for HIV1, 2, HCV, or is at risk for transmitting Creutzfeldt-Jakob disease (CJD). Lookback will also be performed by CTS when notified by the blood center of donors confirmed positive for HBV, HTLV, WNV, Zika, and Ebola viruses and Babesia.

POLICY

I.IDENTIFICATION OF INFECTED DONORS

A. Units implicated in the lookback process are identified by the blood center according to their SOPs. The transfusion service is notified in writing of the units and their shipping date. Notification to external hospitals (non CTS facilities) is completed by the blood center, not the RCRL or CTS.

II.RECIPIENTS PHYSICIAN NOTIFICATION OF POSSIBLE VIRAL/CJD/PARASITIC INFECTION (HIV, HCV, HBV, HTLV, WNV, Zika, Ebola, CJD,Babesia)

A.General Requirements

FDA has specific lookback requirements for some infectious agents, e.g., HIV, HCV, CJD, while others are performed by CTS as being important for patient safety (HVB, HTLV, WNV, Zika, Ebola, Babesia). The CTS Pittsburgh and Chicago physician will identify a transfusion recipient according to records available described in Section IV of this policy. Transfusion Service physician will send the patient's physician a letter or secure e-mail notifying him/her of the lookback. HIV and HCV notifications are sent by certified mail or secure e-mail. The rest of the notification letters are sent by regular mail or secure e-mail.

B.Lookback Notification Process

1.**HIV and HCV lookback process:** A notification letter is sent along with a notification form by certified mail or secure e-mail. The physician must promptly return the enclosed notification form to the transfusion service indicating that they accept responsibility for patient notification. In the case of HIV and HCV, patient notification includes the need for HIV or HCV testing and counseling. If the transfusion service cannot locate the physician, does not receive the notification form from the physician, or the physician refuses to accept responsibility for notification, then the transfusion service is responsible for notifying the patient. This is done by the CTS physician at CTS hospitals. The FDA requires that the process of notification be completed within 12 weeks for HCV and HIV and reasonable attempts should be made. In addition to the physician notification, the facility where the patient was transfused will be notified. This notification will go to the transfusion committee or similar entity at the facility.

2.**HCV lookback notification criteria:** A reactive NAT result serves as one basis for initiating lookback. Since confirmatory testing for HCV (RIBA) became unavailable in 2012, new FDA approved algorithms were developed for HCV notification. Notification of transfusion recipients of prior collections from the same donor is not required when the donor is repeatedly reactive on the anti-HCV screening assay but negative on a mini-pool or individual donation HCV NAT assay and non-reactive on a second anti-HCV screening assay. Transfusion Services must make reasonable attempts to perform the notification within 12 weeks when the donor is repeatedly reactive on the anti-HCV screening assay

and positive on a mini-pool or individual donation HCV NAT assay or negative on the HCV NAT assay and reactive on the second anti-HCV screening assay. Notification for HCV is not required if the donor is deceased.

3. **HIV lookback notification criteria:** When testing for HIV is confirmed positive or NAT positive when the screening test is reactive and further testing is not available, or if under an IND or IDE is exempted for such use by FDA, you must notify transfusion recipients of previous collections of blood and blood components at increased risk of transmitting HIV infection, or the recipient's physician of record or a legal representative or relative if the recipient is a minor, deceased, adjudged incompetent by a State court, or if the recipient is competent but State law permits a legal representative or relative to receive information on behalf of the recipient of the need for recipient HIV testing and counseling (see above), you must make reasonable attempts to perform the notification within 12 weeks after receiving the results of further testing for evidence of HIV infection from the collecting establishment . If the recipient received a transfusion in the hospital and died without ever being discharged from the hospital, you must notify the recipient's physician of record but it is left up to the clinician's medical judgement as to whether or not to inform a family member.

4. **HBV lookback notification process and criteria:** The blood center will notify CTS of components from donors that test confirmed positive for HBV on a current donation (surface antigen positive with positive neutralization and/or positive NAT). CTS will notify the patient's physician in writing by letter or secure email and request that he/she provides follow up to the transfusion service of the patient outcome and/or testing. Follow up physician notification is not required if no response is received. If the patient is deceased, physician notification is not required.

5. **CJD lookback notification process and criteria:** The blood center will notify CTS of components from donors that were found to have CJD, vCJD, suspected vCJD, risk factors for CJD, or if withdrawal is recommended in cases under investigation for vCJD (CJD diagnosis and age less than 55). In those situations, consignee notification could enable the consignee to inform the physician, or other qualified personnel responsible for care of the recipients, so that recipient tracing and medically appropriate notification and counseling may be performed at the discretion of health care providers. CTS will notify the patient's physician in writing by letter or secure email and request that he/she provides follow up to the transfusion service of the patient outcome. Follow up notification is not required if no response is received. If the patient is deceased, physician notification is not required.

For transfusible components from a donor with one family member diagnosed with CJD, or with risk factors for vCJD, suspected vCJD (due to geographic risk deferral, transfusion in the U.K. or in France between 1980 and the present, or due to injection of bovine insulin), per FDA Guidance for Industry, it is not appropriate to conduct tracing and notification of recipients of prior donations.

6. **HTLV I/II lookback notification process and criteria:** The blood center will notify CTS of components from donors that test confirmed positive for HTLV I/II. CTS will notify the patient's physician in writing by letter or secure email and request that he/she provides follow up to the transfusion service of the patient outcome and/or testing. Follow up notification is not required if no response is received. If the patient is deceased, physician notification is not required.
7. **Zika virus lookback notification process and criteria:** The blood center will notify CTS of components from donors that test NAT positive for Zika virus. CTS will notify the patient's physician in writing by letter or secure email and request that he/she provides follow up to the transfusion service of the patient outcome and/or testing. Follow up notification is not required if no response is received. If the patient is deceased, physician notification is not required.
8. **WNV virus lookback notification process and criteria:** The blood center will notify CTS of components from donors that test NAT positive for WNV virus. CTS will notify the patient's physician in writing by letter or secure email and request that he/she provides follow up to the transfusion service of the patient outcome and/or testing. Follow up notification is not required if no response is received. If the patient is deceased, physician notification is not required.
9. **Ebola virus lookback notification process and criteria:** The blood center will notify CTS regarding blood and blood components collected from donors later determined to have Ebola virus infection or disease. CTS will notify the patient's physician in writing by letter or secure email and request that he/she provides follow up to the transfusion service of the patient outcome and/or testing. Follow up notification is not required if no response is received. If the patient is deceased, physician notification is not required.
10. **Babesia lookback notification process and criteria**: The blood center will notify CTS of components from donors that test positive by EIA only or EIA and NAT. CTS will notify the patient's physician in writing by letter or secure email and request that he/she provides follow up to the transfusion service of the patient outcome and/or testing. Follow up notification is not required if no response is received. If the patient is deceased, physician notification is not required.

Appendix III Abbreviations

AABB – American Association of Blood Banks
AE – Adverse Event
AHTRs - Acute Hemolytic Transfusion Reactions
AIS – Abbreviated Injury Scale
ARDS – Acute Respiratory Distress Syndrome
Cc – cubic centimeter
CDC –Center for Disease control and prevention
CPD – Citrate Phosphate Dextrose
CPDA – Citrate Phosphate Dextrose Adenine
CPR – cardiopulmonary resuscitation
CRF – case report form
DSMB – data safety monitoring board
ED – emergency department
ELISA - Enzyme-linked immunosorbent assay
EMS – Emergency Medical Service
FFP – Fresh Frozen Plasma
GSW – Gun shot wound
HR – heart rate
ICF – informed consent form
ICU – intensive care unit
IND – investigational new drug
INR – international normalized ratio
IR – interventional radiology
IRB – institutional review board
IV - intravenous
LAR – Legally Authorized Representative
LTWB - Low Titer Whole Blood
mmHg – millimeter of mercury
MOF – Multiple Organ Failure
NI – Nonsocomial Infections
non-WB – non- Whole Blood
OR – operating room
PI – Principal Investigator
PLT -Platelets
PRBCs – Packed Red Blood Cells
PT – Prothrombin Time
PTT – Partial Thromboplastin Time
SAE – Serious Adverse Event
SBP – Systolic Blood Pressure
SOP – Standard Operating Procedure
TACO – Transfusion Associated Circulatory Overload
TBD – to be determined
TBI – traumatic brain injury
TEG - Thromboelastograph
TRALI – Transfusion Related Acute Lung Injury